Preparing and transforming DH5α electrocompetent cells

Based on the protocol by Schenk and Laddaga (1992).

Preparing the cells:

All centrifugation steps are done at 4°C, 3000xg for 15 min.

1- A 200mL culture was grown to an OD600 of 0.35-0.6 and cells were harvested by centrifugation.

2- Cells were washed 3 times with 10% glycerol: 40mL, 20mL and 10mL in succession.

3- Cells were resuspended in a final volume of about 800µL 10% glycerol, aliquoted in 20µL and stored at -80°C.

Electroporation:

One aliquot per transformation was briefly thawed, mixed with ligation/assembly/plasmid and electroporated using a BTX electroporator at 1800V, 200 Ohm and 25pF. Care was taken to keep the cells as cold as possible by keeping them on ice and pre-chilling electroporation cuvettes.

After electroporation, 1ml pre-warmed SOC or LB medium was added quickly and cells were allowed to recover for 1h at 37°C while shaking. Subsequently, cells were plated on LB agar plates with the appropriate antibiotic in /10 and 9/10 dilution.

Reference:

Schenk, S., & Laddaga, R. A. (1992). Improved method for electroporation of Staphylococcus aureus. *FEMS Microbiology Letters*, *94*(1-2), 133-138.

http://femsle.oxfordjournals.org/content/94/1-2/133.abstract